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Decreasing insulin sensitivity in women induces alterations in LH pulsatility

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1 ABSTRACT

2 **Context:** Obesity is associated with neuroendocrine reproductive alterations and decreased
3 fertility.

4 **Objective:** The objective of the study was to gain insight into the neuroendocrine mechanisms
5 implicated in these alterations.

6 **Design:** The effects on pulsatile LH secretion of 28 days of hyper-caloric diet were studied in
7 lean and regularly cycling female volunteers. ~50% extra calories (3 g sucrose/kg BW/day and
8 1g fat/kg BW/day) were added to their individual daily requirements. Spontaneous and insulin-
9 stimulated LH secretion was recorded on two different days, before and at the end of the caloric
10 load.

11 **Results:** The hyper-caloric diet induced an average weight gain of 2.0 ± 0.3 kg ($p < 0.05$),
12 corresponding to a BMI increase of 0.7 ± 0.1 kg/m² ($p < 0.05$). A concomitant decrease of $11.6 \pm$
13 4.6 % in whole body insulin sensitivity was also observed ($\Delta = -1.6 \pm 0.7$ mg glucose/kg/min;
14 $p < 0.05$). The frequency of spontaneous and insulin-stimulated pulsatile LH secretion was
15 increased by $17.9 \pm 9.0\%$ and $26.5 \pm 9.0\%$ respectively (both $p < 0.05$). Spontaneous LH peak
16 amplitude was decreased by $26.5 \pm 9.0\%$ ($\Delta = -0.7 \pm 0.36$ U/l; $p < 0.05$), a change correlated with
17 insulin sensitivity.

18 **Conclusions:** Short-term weight gain in normal female volunteers induces alterations of LH
19 secretion reminiscent to those observed in obesity. A decrease in insulin sensitivity may
20 constitute a mechanistic link between obesity and its associated neuroendocrine dysfunctions.

21

INTRODUCTION

The activity of the female neuroendocrine reproductive axis is closely associated to nutritional status. This relationship was first established in rodents (1), and then subsequently demonstrated in other animal models and in humans (2-6). In the human, Frisch and McArthur (5) postulated that a minimum amount of body fat is necessary to allow the onset and the maintenance of regular menstrual cycles. Consistently, states of low or insufficient energy availability, such as encountered in anorexia nervosa or overexercising, can also lead to hypothalamic amenorrhea (7-9), and it is generally well accepted that insufficient nutrition negatively impacts the activity of the neuroendocrine reproductive axis.

Weight excess and obesity also appear deleterious for reproduction (10-13). However, compared to weight insufficiency, the mechanisms implicated here are far less understood. The first evidence indicating that excessive nutritional intake could be associated with reduced fertility was a population-based analysis conducted in over sixty countries (14). This work suggested that extreme conditions of either very low or very high caloric intake are both associated with poor reproductive outcome. The hypothesis that excessive weight can impact negatively upon the reproductive function is supported by several subsequent studies. Zaadstra et al. (15) showed in a cohort of 500 healthy women seeking insemination for male infertility that their waist-hip ratio was inversely correlated to their conception rate. Similarly, in two retrospective studies including large numbers of individuals, the relative risk of infertility was found to increase with the BMI of the female, or of both partners (12, 16). Consistently, in a prospective study of 3029 couples with unexplained infertility followed for two years, women with a BMI > 28 kg/m² had a 4% decrease in spontaneous pregnancy rate per unit increase in BMI (13). However, these epidemiologic data do not provide any mechanistic explanation.

In rodents, high-fat fed obese female mice displayed lower fertility than the controls fed a normal chow, a phenotype associated with a down-regulation of hypothalamic GnRH expression (17). In humans, polycystic ovary syndrome (PCOS) is an endocrine condition associating overweight or obesity with a reproductive phenotype characterized by dysfunctions at the neuroendocrine and the ovarian levels (18-20). Peripheral insulin resistance, a hallmark of PCOS, has been implicated in the pathogenesis of the syndrome. Insulin, like leptin (21, 22), is a metabolic signal involved in the central nervous system regulation of body weight and

reproduction (23-25). This dual function is illustrated by the phenotype of NIRKO (for Neuron-specific Insulin Receptor Knock Out) mice, which harbor a neuron-specific deletion of the insulin receptor gene (23). These mice have complete insulin resistance in the central nervous system, and develop hyperphagic obesity associated with hypogonadism of hypothalamic origin. The latter observation suggests that in mice, activation of GnRH neurons is dependent upon adequate insulin signaling within the hypothalamus. This hypothesis is consistent with our previous *in vivo* (26) and *in vitro* data demonstrating that in rodents, hypothalamic GnRH neurons are indeed insulin-sensitive (27, 28). Since we could also demonstrate that in normal young women, pulsatile secretion of LH is modulated by insulin (29), we hypothesized that insulin could play a pathophysiological role in the reproductive phenotype of PCOS.

Therefore, the aim of this study was to further explore the interaction between insulin and the control of reproduction in humans. We used a model of short-term hyper-caloric diet to test the hypothesis that changes in whole body insulin sensitivity can modify the secretion of neuroendocrine reproductive hormones in humans. Given the close association between obesity and insulin sensitivity, a better understanding of the relationship of the latter with fertility could become very important in the work-up and treatment of obesity and related metabolic disorders.

MATERIALS AND METHODS

Study subjects.

The study was approved by the local Ethics Committee and all volunteers provided written informed consent. Subjects were recruited by poster campaign. Between June 2013 and February 2014, 107 potential subjects were evaluated by telephone screenings, 27 of which were identified as eligible for the screening visit, and thirteen finally included in the study. All anthropometrics measurements were recorded at 07:30 am on the test days, after an overnight fast. Height was measured at the screening visit, and body weight was assessed at each visit.

Four subjects discontinued the study for the following reasons: irregular menstrual cycles (n=1), failure to adhere to the protocol (n=2) and occurrence of hypokalemia during insulin infusion (n=1). Thus, nine subjects completed the study and were included in our analyses. After completing the study, all the volunteers were offered dietary consultations to help them restore their baseline weight.

Study design

A hypercaloric feeding protocol lasting 28 days was used to explore the effects of weight gain and decreased whole body insulin sensitivity on neuroendocrine reproductive hormones. To this end, pulsatile luteinizing hormone secretion was used as a surrogate marker of the activity of hypothalamic GnRH neurons (30, 31). The spontaneous and the insulin-stimulated LH secretion profiles were evaluated twice: the first time under strict conditions of controlled isocaloric diet, and the second time at the end of 28 days of hypercaloric feeding. The evaluations of LH secretion were always performed in the follicular phase of the menstrual cycle, according to our previously published protocols (29).

Nutritional interventions.

The controlled isocaloric diet was initiated three days before the anticipated first day of the next menstrual cycle, and was designed to last until completion of the two test days described below (approximately seven days, depending upon the occurrence of menstruations). The

energetic content of the isocaloric diet was calculated to provide 1.5 times the resting metabolic rate measured by indirect calorimetry (32, 33). The nutritional composition of the isocaloric diet was 55% carbohydrates, 15% proteins and 30% fat.

The hypercaloric diet was designed to last 28 days in total, and was initiated 24 days before the expected day of the next menstrual cycle. During the initial 21 days of voluntary high calorie intake, subjects were instructed to add a supplement of sucrose (3g/kg/day, diluted in 1L of water) and lipids (1g/kg/day, 2/3 butter and 1/3 olive oil) to their *ad-libitum* diet. Three days before the expected day of the next menstrual cycle, they were switched for an additional seven days to controlled hypercaloric feeding, consisting in an isocaloric diet supplemented with 3g/kg/day of sucrose and 1g/kg/day of lipids. During that period, we provided all necessary food to the volunteers, who were asked to restrain from eating anything else. Controlled feeding was adopted during the last week of the intervention in order to normalize the nutritional intake at the time of the tests. Also, volunteers were asked to avoid all strenuous physical activity during the entire overfeeding period. The two nutritional interventions (isocaloric and hypercaloric feeding) were separated by at least one month.

Test days

At the end of each intervention (isocaloric or hypercaloric feeding), volunteers were admitted twice to the Clinical Research Center of the University hospital of Lausanne, the first time for a “clamp day” and the second time for a “fasting test day”. All test days consisted in frequent (every 10 minutes) blood sampling and were started at 07:00 am with the admission of the volunteer after an overnight fast. On the fasting days, a single catheter was inserted into a forearm vein, and frequent blood sampling was started after 30 minutes of rest. The total duration of the protocol was ten hours, during which volunteers were kept fasted. Body composition was evaluated by bioimpedance analysis (Imp DF50, ImpediMed) during the day.

For the clamp days, a second catheter was inserted into a contralateral forearm vein to infuse insulin and glucose. During clamp days, subjects underwent the same frequent blood sampling protocol as the fasting day, this time together with a hyperinsulinemic euglycemic clamp lasting throughout the ten hours of sampling. Clamp days were performed first, between day 2 and 7 of the menstrual cycle, and fasting days took place exactly three days later. The order of the test

days was not randomized, to avoid programming a full day of fasting before the clamp. All blood samples were immediately centrifuged upon collection, and serum was frozen onsite.

Hyperinsulinemic and euglycemic clamps were performed according to our previously published protocol (29). A bolus of insulin was injected thirty minutes after the insertion of the catheters, followed by a constant infusion at a rate of 1 mU/Kg/min, which was continued for ten hours. Blood samples were obtained every five minutes for the assessment of glycemia, using two different measures obtained onsite on a glucometer (Accu-chek Aviva, Roche). A variable infusion of 20% glucose allowed clamping of glycemia at 5.5 mmol/L (34).

Assays and data analysis

LH, FSH and insulin were measured by immunoenzymologic assay (COBAS, Roche Diagnostics International AG, Rotkreuz, Switzerland). Leptin and grehlin were measured by ELISA and adiponectin by multiplex analysis, using commercially available kits and reagents (Merck Millipore AG, Schaffhausen, Switzerland). Oestradiol, progesterone, and testosterone were measured by chemiluminescent microparticle immunoassay (Architect i2000SR, Abbott AG, Baar, Switzerland). Glucose was measured by hexokinase, cholesterol by cholesterol-oxidase and p-amino-antipyrine (CHOD-PAP), HDL cholesterol by CHOD-PAP, homogeny polyethylene glycol, LDL cholesterol calculated with Friedewald formula, hsCRP by immunoturbidimetry and triglycerides by glycerol phosphate oxidase PAP (all on COBAS, Roche Diagnostics International AG, Rotkreuz, Switzerland).

The characteristics of LH pulsatility were analysed by a modified Santen and Bardin method (35). LH pulse amplitude was calculated as the difference between the nadir and the highest peak within 30 minutes of the nadir. Pulse amplitudes during a given admission were averaged for each subject. Whole-body insulin sensitivity was expressed as the mean rate of glucose infusion necessary to maintain euglycemia for the last eight hours of the clamps. HOMA-IR was calculated using the standard formula (36). Statistical analyses were completed using Wilcoxon's nonparametric signed-rank test for the between group comparison and the nonparametric Spearman's correlation method for the correlation analysis. Power analyses were completed using SAS Power & Sample Size software (SAS Inc, Cary, NC, USA).

RESULTS

Study subjects and design

The subjects were women aged between 18 and 30 years (mean age 23.6 ± 0.8 y), with regular menstrual cycles of 30.2 ± 0.8 days and a mean BMI of 21.9 ± 0.7 kg/m² (mean weight of 60.5 ± 2.2 kg). Their mean body fat content was 24.1 ± 2.5 %. Mean fasting glycemia was 4.6 ± 0.1 mmol/L, with a mean fasting insulin of 5.2 ± 0.7 mU/L and a mean HbA1c level of 5.3 ± 0.1 %. These parameters were assessed at the screening visit between day 4 and day 9 of the menstrual cycle and confirmed that all the variables were within the normal range at baseline. In addition, they were not pregnant, did not take hormonal contraception, were not participating in sport for more than four hours a week, did not smoke more than ten cigarettes per day and did not drink more than three alcoholic beverages per day. A complete medical history did not identify any hormonal problem, and physical examinations were unremarkable.

The individual duration of the dietary intervention was somewhat variable, depending upon the length of the menstrual cycle. Thus, the controlled isocaloric diet lasted 7.1 ± 0.7 days, the average time interval between the isocaloric and the hypercaloric phases was 62.5 ± 11 days, and the length of the hypercaloric phase (voluntary overfeeding + controlled hypercaloric diet) was 29.4 ± 0.8 days, of which 7.6 ± 0.6 were controlled diet.

Metabolic data

The hypercaloric diet induced a mean weight gain of 2.0 ± 0.3 kg, with most participants returning to their baseline weight by the final visit (Figure 1A). The subject with the lowest baseline BMI gained the least amount of weight (BMI = 18.6 kg/m²; Δ weight = 0.7 kg), whereas the subject with the highest baseline BMI gained the most weight (BMI = 24.9 kg/m²; Δ weight = 4.0 kg, Figure 1B). Of note, the latter subject was also the only participant who had not returned to her inclusion weight by the final visit. The evolution of the volunteers' body mass index followed the same pattern, with the hypercaloric diet inducing a mean increase of 0.7 ± 0.1 kg/m² ($p < 0.05$). We also found a significant increase of 3.8 ± 1.5 kg in absolute fat mass at the end of the hypercaloric diet ($p < 0.05$, Figure 1C), translating into a relative increase of 5.2 ± 2.1 % ($p < 0.05$).

These changes in body weight and composition were accompanied by a significant decrease in whole body insulin sensitivity. The mean glucose infusion rate necessary to maintain euglycemia during the insulin clamp performed in isocaloric conditions was 12.1 ± 3.0 mg/kg/min, compared to 10.4 ± 2.4 mg/kg/min at the end of the hypercaloric diet. The latter value, albeit still within the normal range, was significantly lower than the former ($\Delta = -1.57 \pm 2$ mg/kg/min ; $p < 0.05$). Insulin resistance according to the homeostatic model assessment (HOMA-IR) also increased significantly between the isocaloric and the hypercaloric phase (1.1 ± 0.2 vs 1.5 ± 0.2 , respectively; $\Delta = 0.4 \pm 0.1$; $p < 0.05$). Evaluations of circulating metabolites showed that total cholesterol, HDL cholesterol, fasting glucose and insulin levels all increased significantly with the hypercaloric diet (Supplemental table). Leptin and adiponectin were also significantly increased by hypercaloric feeding, whereas ghrelin was not affected (data not shown).

Menstrual cycle & reproductive hormones.

The clamp test days took place during the early follicular phase (day 4.3 ± 0.5 of the cycle in isocaloric condition, and 5.0 ± 0.4 in hypercaloric condition). The fasting test days, programmed three days after the clamps, occurred significantly later in the cycle (day 7.3 ± 0.5 in isocaloric condition, and 8.0 ± 0.4 in hypercaloric condition). The occurrence of a progression in the cycle between the two test days is also supported by the rising levels of LH and estradiol observed in the isocaloric phase (Table 1).

LH secretion profiles.

Figure 2 displays the spontaneous LH secretion profile of each volunteer at the end of isocaloric and hypercaloric feeding. In eight of the nine volunteers, the LH pulse frequency on the isocaloric fasting test day was between six and nine peaks in ten hours. A single volunteer (#8) had only one LH peak. At the end of hypercaloric feeding, LH pulse frequency was increased in six individuals, unchanged in two and decreased (from eight to seven in ten hours) in a single individual (#2). Similarly, mean LH pulse amplitude was between 0.83 and 2.43 U/L for all volunteers except subject #8 who had a single peak with an amplitude of 5.2 U/L. At the end of hypercaloric feeding, mean LH pulse amplitude was decreased in all subjects but one (#9) in whom the amplitude increased from 2.0 to 2.46 U/L. Of note, subject #9 was also the only subject showing an increase in whole body insulin sensitivity at the end of the hypercaloric diet.

Figure 3 displays insulin-stimulated LH secretion profiles of each volunteer before and after hypercaloric feeding. Similar to that which is observed for spontaneous LH secretion, LH peak frequency was increased in seven individuals at the end of hypercaloric feeding, and remained unchanged for the remaining two subjects (#1 and #7). Mean LH pulse amplitude was decreased at the end of hypercaloric feeding period in seven out of nine volunteers. Exceptions were volunteer #8 and #9.

Figure 4 summarizes the changes occurring in pulsatile LH secretion. At the end of the hypercaloric diet, the spontaneous LH pulse frequency (panel A) was increased by $17.9 \pm 9.0\%$ ($p \leq 0.05$), while the amplitude of pulses (panel C) was decreased by $26.5 \pm 9.0\%$ ($p \leq 0.05$). These changes in LH pulse amplitude were positively correlated with the changes occurring in whole body insulin sensitivity (Figure 5). LH pulse frequency was also increased by $29.9 \pm 10.2\%$ during the insulin clamp performed at the end of the hypercaloric diet (Figure 4, panel B, $p \leq 0.05$). Finally, mean LH levels were not affected by the intervention.

DISCUSSION

Obesity has been associated with alterations in neuroendocrine reproductive hormones that have been linked to impaired fertility (37), but little is known regarding the mechanisms implicated. In order to investigate the role of peripheral insulin resistance in this context, young and lean women with normal menstrual cycles were studied before and at the end of one month of administration of a hypercaloric diet. This high sugar, high fat diet, calculated to attain ~150% of their daily energy requirements, induced significant increases in body weight, decreases in whole body insulin sensitivity and increases in fasting insulin levels. Patterns of pulsatile LH secretion were significantly altered at the end of hypercaloric feeding, when compared to the isocaloric diet. Changes consisted in an increased frequency of pulsatile LH secretion with lower amplitude pulses, both in spontaneous and insulin-stimulated conditions. Of note, the respective direction of these changes is entirely coherent with these two parameters of LH pulsatility being somewhat interdependent, adding to the robustness of our observations.

A modulation in the frequency of pulsatile LH secretion cannot occur at the pituitary level. Therefore, the increase observed in LH pulse frequency is likely a reflection of a hypothalamic effect of our intervention (38, 39). Rodent data showing an acceleration of the frequency of pulsatile GnRH secretion from hypothalamic explants obtained from diet-induced obese (DIO) mice suggest that this effect could be mediated by GnRH neurons (40). In contrast, the origin of the decrease in LH peak amplitude could be either at the hypothalamic or at the pituitary level (38). Indeed, a smaller peak amplitude could result from a stimulation by lower concentrations of GnRH, reflecting decreased hypothalamic GnRH secretion. However, it could equally result from a modification of the sensitivity of the pituitary gonadotroph cells to GnRH stimulation, as previously reported in another model of DIO mice (41). Although we found no overall effect of hypercaloric feeding on LH pulse amplitude during the insulin clamps, changes were observed at the individual level. LH pulse amplitude was indeed decreased during the clamps performed at the end of the period of hypercaloric feeding in seven of the nine volunteers. The two exceptions were volunteer #8, who displayed no peak at all during the isocaloric clamp test day, and volunteer #9, who experienced an increase in insulin sensitivity at the end of the hypercaloric diet. Thus, the increase in mean peak amplitude of volunteer #9 is entirely consistent with the positive correlation observed between spontaneous LH pulse amplitude and whole body insulin sensitivity.

These data confirm the existence of an important link between insulin and the control of LH secretion in humans (29), possibly related to the long term elevations in peripheral insulin levels occurring in states of insulin resistance. Indeed, one can hypothesize that the decreases in whole body insulin sensitivity observed at the end of hypercaloric feeding are not paralleled by similar decreases at the level of the CNS, rendering the hypothalamo-pituitary unit highly sensitive to these raised insulin levels. This hypothesis is entirely consistent with rodent data, where alterations in the neuroendocrine reproductive hormones similar to those reported here are induced by changes in insulin signaling within GnRH neurons as well as pituitary gonadotroph cells (40, 41).

All the volunteers experienced weight gain at the end of the hypercaloric diet, resulting essentially from an increase in fat mass. Thus, our intervention induced modifications in body composition very similar to what is usually observed in more chronic conditions of excessive energy intake, together with a number of changes in blood markers. In particular, fasting glycemia, fasting insulinemia and the insulin resistance index HOMA-IR were all increased at the end of the hypercaloric diet. As stated above, whole body insulin sensitivity measured during the hyperinsulinemic euglycemic clamp studies was also significantly decreased at the end of the hypercaloric diet, although it clearly did not reach values typically associated with insulin resistance such as those seen in type 2 diabetes mellitus.

These metabolic modifications are nevertheless physiologically significant, again reminiscent of the early stages of obesity. Interestingly in this context, decreases in LH pulse amplitude have been reported in obese women (42). The present data demonstrate that short-term hypercaloric feeding of normal volunteers can reproduce some of the neuroendocrine modifications associated with excess weight of much longer duration. They also demonstrate that these alterations appear early in states of excessive caloric intake in humans, during the phase of dynamic weight gain, validating further the use of this model in the study of human obesity.

As previously reported (43-45), adiponectin was higher at the end of the hypercaloric feeding period, confirming the existence of a differential regulation between short and long-term conditions of positive energy balance (46, 47). We also observed a large rise in leptin levels after hypercaloric feeding, consistent with the increase observed in fat mass. Given the well-known actions of leptin in both the regulation of energy intake and body weight and of the

neuroendocrine reproductive system, leptin was a logical candidate link between metabolic changes and the hypothalamic GnRH pulse generator in our study. However, we did not observe any significant correlation between parameters of LH secretion and leptin levels. Thus, the question of the implication of leptin in conveying signals of excessive energy intake to the hypothalamic GnRH pulse generator remains open.

In conclusion, we could demonstrate that 28 days of hypercaloric feeding in normal female volunteers can induce alterations in the activity of the neuroendocrine reproductive axis that are reminiscent of the changes reported in more chronic states of obesity. Thus, our data suggest that these alterations may take place at very early stages of the disease. By showing changes in both the frequency and the amplitude of LH secretion pulses, our data also suggest that this modulation is occurring both at the hypothalamic and the pituitary levels. Moreover, the existence of a significant correlation between changes in whole body insulin sensitivity and these hormonal alterations is providing for the first time a mechanistic explanation linking the neuroendocrine dysfunctions associated with calorie overload and obesity to insulin signaling. Further work in infertile obese patients will be necessary to better delineate the therapeutic implications of our observations.

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| | IC | IF | HC | HF | <i>P</i> ¹ | <i>P</i> ² |
|-----------------------|-------------|-------------|-------------|-------------|-----------------------|-----------------------|
| Day of cycle | 4.3 ± 0.5 | 7.3 ± 0.5 | 5 ± 0.4 | 8 ± 0.4 | <0.01 | <0.01 |
| Oestradiol (nmol/l) | 0.13 ± 0.01 | 0.17 ± 0.03 | 0.16 ± 0.01 | 0.22 ± 0.03 | <0.05 | ns |
| LH (U/l) | 3.53 ± 0.54 | 5.81 ± 0.66 | 5.31 ± 0.43 | 6.36 ± 0.57 | <0.05 | ns |
| FSH (U/l) | 4.91 ± 0.4 | 5.26 ± 0.38 | 5.4 ± 0.52 | 5.46 ± 0.42 | ns | ns |
| Testosterone (nmol/l) | 0.87 ± 0.06 | 0.97 ± 0.07 | 0.88 ± 0.08 | 1.01 ± 0.08 | ns | ns |

Table 1: timing of the various test days with respect to the menstrual cycle (day 1 defined as the first day of menstruations), with the baseline levels of reproductive hormones. *P*¹ indicates a difference between IC and IF and *P*² indicates a difference between HC and HF.

Data expressed as mean ± SEM.

IC: isocaloric clamp day; IF: isocaloric fasting day; HC: hypercaloric clamp day; HF: hypercaloric fasting day.

| | Isocaloric phase | Hypercaloric phase | <i>P</i> |
|------------------------|------------------|--------------------|----------|
| Glucose (mmol/L) | 4.85 ± 0.11 | 5.05 ± 0.09 | <0.05 |
| Insulin (mU/L) | 5.09 ± 0.82 | 6.59 ± 0.8 | <0.05 |
| Cholesterol (mmol/L) | 3.24 ± 0.19 | 3.60 ± 0.15 | <0.05 |
| HDL chol (mmol/L) | 1.33 ± 0.12 | 1.49 ± 0.10 | <0.05 |
| LDL chol (mmol/L) | 1.63 ± 0.15 | 1.83 ± 0.13 | ns |
| Triglycerides (mmol/L) | 0.62 ± 0.05 | 0.61 ± 0.04 | ns |
| hsCRP (mg/L) | 0.00 ± 0.00 | 1.22 ± 0.52 | n/a |

Supplemental table : metabolic profiles (fasting) at the end of the iso-caloric and the hyper-caloric phases, respectively.

Data expressed as mean ± SEM.

FIGURE LEGENDS

- Figure 1: Weight and body adiposity. Panel A: weight of the volunteers at the different time points indicated. Panel B: evolution of individual weights of the nine volunteers. Panel C: fat mass before and at the end of hyper-caloric diet. I: inclusion; IC: isocaloric clamp day; IF: isocaloric fasting day; HC: hypercaloric clamp day; HF: hypercaloric fasting day; FV: final visit. * $P < 0.05$. Data represented as median, 10th, 25th, 75th and 90th percentile in panels A and C.
- Figure 2: Individual profiles of spontaneous LH secretion during IF (left) and IC (right). Open circles indicate significant peaks. IC: isocaloric clamp day; IF: isocaloric fasting day.
- Figure 3: Individual profiles of insulin-stimulated LH secretion during HF (left) and HC (right). Open circles indicate significant peaks. HC: hypercaloric clamp day; HF: hypercaloric fasting day.
- Figure 4: Characteristics of pulsatile LH secretion. Panels A and B: LH peak frequency. Panels C and D: LH peak amplitude. Panels E and F: mean LH levels. * $P < 0.05$. IC: isocaloric clamp day; IF: isocaloric fasting day; HC: hypercaloric clamp day; HF: hypercaloric fasting day. Data represented as median, 10th, 25th, 75th and 90th percentile.
- Figure 5: Positive correlation between LH pulse amplitude and whole body insulin sensitivity. r obtained by nonparametric Spearman's correlation test.









